Allogeneic human umbilical cord-derived mesenchymal stem/stromal cells for frailty: a randomised phase I/II trial.

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Abstract

Frailty, a specific condition of increased vulnerability and reduced general health associated with aging in elderly people, is an emerging global burden requiring major implications for clinical practice and public health. The lack of standardized definition and treatment of the disease resulted in the increasing number of elder diagnosed with frailty. Recently, preclinical and clinical studies support the safety of mesenchymal stem/stromal cells (MSCs) in the treatment of frailty. However, no comprehensive study has been conducted to access the interrelationship between frailty condition and the effects of MSC-based therapy. To fill the gap of knowledge, the aim of this trial is to investigate the safety and potential therapeutic efficacy of allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) in combination with standard frailty treatment in Vietnam. Moreover, this study describes the rationale, study design, methodologies and analysis strategy currently employ in stem cell research and clinical study. This randomized case-control phase I/II trial is conducted at Vinmec Times City International Hospital, Hanoi, Vietnam between May 2021 and September 2022. In this trial, 44 patients will be enrolled and randomized into a UC-MSC administration group and control group. Both groups will receive the standard frailty treatment and supplementary medication. The UC-MSC group will received two doses of thawed UC-MSC product at 1.5×10^6 cells/kg patient body's weight with an intervention interval of three months. The primary outcome measures will include the incidence of prespecified administration-associated adverse events (AEs) and serious adverse events (SAEs). The potential efficacy will be evaluated based on the improvement in frailty conditions (including physical examination, patient-reported outcomes, quality of life, immune markers of frailty, metabolism analysis, and cytokine markers from patient's plasma). The clinical evaluation will be conducted at baseline and 3, 6 and 9 months post-intervention.

Introduction

Frailty as a new frontier of medicine

Frailty, a specific condition of increased vulnerability and reduced general health associated with aging in elderly people, is an emerging global burden requiring major implications for clinical practice and public health (1). In countries with ageing population including Vietnam, the prevalence of frailty increases rapidly (2). The estimated prevalence greatly varies between countries and ranging between 4 to 59% due to non-standardization of frailty definition and evaluation (3). In Vietnam, a study of 461 patients at National Geriatric Hospital in Hanoi indicated that the prevalence of frailty was 31.9% according to Reported Edmonton Frail Scale (REFS) (4). Patient diagnosed frailty is characterized by a decline in functioning in multiple organs and the increasing vulnerability to stressors. Frailty individual usually faces with increased mortality, hospitalization, falls, and admission to long-term care (5).

Although there is no standardized definition of frailty, the three important factors associated with the disease have remained consistent over the past decades (6). First, frailty is a multidimensional condition associated with physical and psychological factors. Second, frailty is an extreme condition of ageing process. Third, patient diagnosed with frailty can fluctuate between different severity levels of frailty during their elderhood (1). In 2001, a widely accepted clinical description of the disease proposed by Fried and colleagues, including five major criteria, including (1) unintentional weight loss, (2) weak grip strength, (3) low gait speed, (4) low physical activities, and (5) exhaustion (7). Based on these criteria, the patient exhibits one to two symptoms is classified as pre-frailty whereas the patient shows at least three criteria is diagnosed frailty (Figure 1).

To manage the consequences of frailty in response to population ageing, numerous interventions are introduced, including physical activity, nutritional control via protein-calorie supplementation, and de-prescription of unnecessary medications (8, 9). However, the lack of standardized guideline for frailty treatment resulted in the variations of treatment efficacy across the globe. In fact, the effectiveness of these intervention is not supported by a solid evidence from trials (10). Hence, it required accurate and more evidence-based knowledge regarding which intervention strategies are effective for frailty, and ascertain whether they are applicable in developing countries, less labor intensive, cost-effective and reproducible in low-income world. Moreover, because frail people are undergone different stages of frailty during

their life-span, called dynamic state transition (11), it is important to deploy the managing strategy that allows performing clinical care across the continuum of frailty. Thus, looking for an effective treatment to prevent or help the old people recovery from frail condition emerges as a trend in regenerative medicine recently (12).

Regenerative medicine in treatment of Frailty

Medical advancement and improvement in healthcare service have contributed to a longer and better life quality of frail people. However, as the growing proportion of aging population, the number of frail elderly patients is increased gradually resulted in the need for healthcare supports. Recently, the regenerative medicine, especially mesenchymal stem cell (MSCs) therapy, emerges as an alternative candidate for frailty as there are specific nature of frailty syndromes that support the mechanism of action of MSCs (12).

Oxidative stress and inflammatory reaction occurs spontaneously along the aging process result in an alteration at molecular and cellular level (Figure 1) (13). Lifestyle conditions including low physical activity, unhealthy diet, inadequate nutritional habits together with genetic susceptibility and background chronic disease promote the oxidative stress and inflammation to an extreme level as found in frail patients. Therefore, frailty is strongly associated with oxidative stress and inflammation along aging process, which includes the following major hallmarks: (1) instability of genomic materials, (2) reduction of telomerase activities and telomere attrition, (3) loss of proteostasis, (4) reduction of nutrient-sensing, (5) metabolism malfunctions (including mitochondrial dysfunction), (6) cellular senescence, (7) stem cell depletion, and (8) alternation of cell-to-cell communication (Figure 1) (14). These aging hallmarks play a significant role in the development of other geriatric syndromes once frailty is established and progresses in its natural courses, including cardiovascular disease, hypertension, arthritis, diabetes, etc (15). Moreover, these aging-related features of frailty are also altered the endogenous stem cell regeneration and function which, in turn, reduced the regenerative capacity of multiple organs and tissues. Toward this end, providing an exogenous stem cell population in order to replenish the stem cell pools and improved the regenerative ability emerges as an alternative and promising approach for frailty (16).

Among other sources and types of stem cells currently available, mesenchymal stem/stromal cells (MSCs) are the cell source of choice due to their ability to migrate to injury site to regulate the immune response, reduce inflammation and promote cellular repair (17). Notably, since it first discovered in 1960s, MSCs have been widely used and proven to be

partially effective in numerous diseases, including autism (18), cerebral palsy (19), spinal cord injury (20), chronic obstructive pulmonary dysplasia (21), bronchopulmonary dysplasia (22), diabetes and cardiovascular disease (23). MSCs improve outcomes in patients with these diseases by reducing inflammation responses, reduce TNF-α and CRP levels, and are reported to be safe in-patient regardless of age (24, 25). MSCs possess the ability to evade and regulate the host's immune system due to the lack of major histocompatibility complex (MHC)/human leukocyte antigen (HLA) class II and associated costimulatory molecules and low level of HLA-DR (26). The interplay between MSCs and the immune defense system is believed to be diverse via different pathways. MSCs suppress the proliferation and maturation of both B- and T-lymphocytes in paracrine manner via secreted factors and via cell-to-cell contact (27, 28). MSCs reduce the expression of proinflammatory cytokines, such as TNF-α, IL-2, IL-1β, IL-6 and CRP. The interaction between the hosts' immune system and MSCs' activities is interconnection. While MSCs can alter the response of immune cells, the host immune system can also modulate and stimulate the actions of MSCs. The elevated level of cytokines and chemokines at the injury sites, such as interferon-γ (IFN-γ) triggers the induction of HLA class I and II on the MSC surface (29, 30). Whereas reduction of IFN-γ and TNF-α was reported to switch MSCs to release pro-inflammatory cytokines (31, 32).

Accumulation of senescent cells is one of the hallmark of human aging that is tightly related to the telomere maintenance and DNA damage. The former has a profound impact on cell proliferation and senescent because the shortening of telomere has been suggested as a useful biomarkers for cellular senescence and aging (33). Although the interrelationship between telomere shortening and frailty has been suggested to be a potential biomarker of frailty in clinical level, no correlation between telomere length and frailty condition has been reported (34, 35). In response to persistent DNA damage, cellular senescent occurs through the activation of the INK4a/ARF (CDKN2a) locus leading to increase expression of p16INK4A – a cell cycle kinase inhibitor. Therefore cellular senescence can be measured by level of expression of the p16INK4A. In fact, studies have revealed that p16INK4a correlate with chronological age in both mice and human and peripheral blood T-lymphocyte expression of p16INK4A has been established as an indicator of human aging (36). A meta-analysis of more than 300 genome wide association study identify that INK4a/ARF locus is generally linked to the highest number of age-associated pathologies such as cardiovascular diseases, diabetes, glaucoma and Alzheimer's disease (37). In frail elderly, MSC might have benefits in anti-aging

via multiple mechanism as described above, it is assumed that MSCs might reduce cellular senescence in these people.

To date, at least four clinical trials have been conducted to evaluate the safety and efficacy of MSC therapy in treatment of frailty. Among them, two clinical trials (registered number NCT02982915 and NCT03169231) are ongoing multicenter, randomized, blinded, and placebo-controlled clinical studies using bone marrow-derived MSCs (38). Another trial, named CRATUS (NCT02065245), was completed recently by Joshua M. Hare and colleagues in 2020 (39). The results confirm that all participated patients are well-tolerated to the allogeneic administration of BM-MSCs. In terms of efficacy, the trial indicated that 100-million cell doses showed a more effective treatment than 200-million cell doses (40). Especially, the TNF-α (an important biomarker associates with inflammation and immunity) level reduced significantly after 6-months post-administration. Other frailty examinations, such as 6-minute walk distance test (6MWT), exhaustion-multidimensional fatigue inventory (MFI), and C-reactive protein (CRP) levels did not give consistent results or did not reach statistical significant post-administration.

The need for allogeneic MSC therapy in frailty treatment

Because of no standard and effective treatment for frailty elders, applications of regenerative medicine to replenish the endogenous stem cell pool, regulate inflammation and immune stressor, reduce age-related dysfunction of multiple organs, and enhance regenerative function of patient is an alternative approach for frailty (41). Autologous MSCs (auto-MSCs) have not been used for frailty in clinics. It is because autologous approach requires an invasive method to obtain either bone marrow or adipose tissue from frail patient posing the risk of medical complication and infection and treatment is available only after a delay of two to three weeks ex vivo expansion period to achieve the targeted cell dose for administration. Moreover, autologous MSCs are strongly affected by aging causing the reduction in their therapeutic functions (42, 43). Compare with auto-MSCs, allogeneic MSCs (allo-MSCs) have great advantages for establishment of readily available, disease-free cell products, and non-invasive approach for frailty treatment, important features for disease associated with fragile and vulnerable patients.

Although the preclinical and currently finished clinical trials supported a promising future of frailty treatment using MSC therapy, several outcome measurements are variable with controversial results. Recently report from clinical trial indicates a preliminary outcome of

BM-MSCs with non-statistical significant results (40). This is compensated by the following trial conducting in larger cohort (120 patients, NCT03169231) providing more evidence-based study to support the efficacy of BM-MSCs. In the CRACTUS study, BM-MSCs were isolated and expanded from bone marrow aspirate from male or female donor between the ages of 20 to 45 with a comprehensive screening history and physical status (39). Once collected, the BM-MSCs were expanded in 20% Fetal bovine serum (FBS) supplemented media for 14 days and harvested at passage 1 for administration. Based on the nature of FBS-cultured MSCs and the BM-MSCs themselves, several limitations might be present and need to be discussed. *In vivo* evidence suggested that BM-MSCs can be affected by aging and strongly associated with mammalian life span and health condition (44, 45). Recently, our group reported the negative effects of type 2 diabetes mellitus duration on the quality and metabolic function of autologous BM-MSCs. It is proposed that BM-MSCs react to environmental and hosts' physical conditions in response to age-related stimuli could perpetuate aging and associated with age-related senescence and disease. Moreover, *in vitro* expansion of BM-MSCs compromises the biological characteristics of BM-MSCs and their senescence with prolonged culture (46). Hence, the variation in the efficacy of recently study using BM-MSCs in treatment of frailty could be due to: (1) heterogeneous sources of BM-MSCs derived from wide age range of BM-MSCs' donors, (2) *in vitro* culture of BM-MSCs in FBS (unknown component, batch-to-batch variation, and animal-derived products), and (3) aging-related effects of BM-MSCs.

An alternative source of allo-MSCs is needed in order to fill a gap in knowledge and provides another option regarding the strengths and limitation of BM-MSC therapy as a source of stem cells for treatment of frailty using regenerative medicine. Therefore, in this study, we propose a matched case-control phase I/II trial using allogeneic administration of umbilical cord-derived MSCs (UC-MSCs) for frailty. The primary goal is to evaluate the safety of UC-MSC administration in elderly patients diagnosed frailty and identify factors associated with allogeneic UC-MSC administration (such as immunological response, D-dimer level, and thromboembolism). The secondary outcome is to access the efficacy of allogeneic UC-MSC administration for frailty treatment via absolute change of five important clinical examination of frailty, including patient body weight, 6MWT, functional status, quality of life, pulmonary function. Additionally, in this study, the interlink between stem cell therapy and treatment's safety and efficacy is evaluated via several experiments, including stem cell metabolic analysis, immunoregulatory examination, cellular senescence and expression of tissue factor (CD142). Completion of this study not only provides more evidence-based observation in the application

of MSC therapy for frailty but also sheds a light into the mechanisms by which stem cell therapy could be further enhanced their safety profiles and improved their efficacy for frail elderly patients.

Study Description

Study objectives

The aim of this trial is to evaluate the safety and potential efficacy of allogeneic UC-MSC administration in patients with frailty. There are three specific objectives:

1. Evaluate the safety of intravenously (IV) administered UC-MSC in patients with frailty.
2. To access the potential efficacy of the treatment.
3. To explore the potential therapeutic mechanism of UC-MSCs in the treatment of frailty.

Study design, ethics, and dissemination

This randomized controlled phase I/II clinical trial was approved by the Ethical Committee of Vinmec International Hospital (number: 75/2021/QĐ-VMEC) and The National Ethical Board (number: 111/CN-HĐĐĐ). This study was registered at ClinicalTrials.gov (number NCT04919135). A total of 44 patients with frailty will be recruited at the Regenerative Medicine Department at Vinmec Times City International Hospital, Hanoi, Vietnam, between May 2021 and September 2022. We will disseminate the research results through high-quality peer-reviewed open access (via PubMed) journals and presentations at national and international conferences. Finally, an ongoing update of the trial will also be provided and shared annually with our partners in the health system and community agencies according to National Regulation.

Sample size

As a previous study indicated that the score of Community Healthy Activities Model Program for Seniors (CHAMPS) of patients was $5118,5 \pm 1049,9$. We set this indicator at 20% reduction to calculate the minimum sample size for the proposed study. According to the continuous endpoint, two Independent sample study, we assumed α was 0.05 and power was 85%. Thus, the smallest sample size was 44 patients. The calculated sample size was 22 for each group.

Randomization

All patients will be randomized (1:1) into either the UC-MSC administration group (n=22) or the control group (n=22) (Supplementary Figure 1). Patients from both groups will receive a standard medication treatment according to the Vietnamese Ministry of Health guidelines, which includes the use of Hightamine (Hankook Korus Pharm, Korea), total calcium (Nugale Pharmaceutical, Canada), Bioflex (Ausbiomed, Australia) and Nootropil (UCB Pharma S.A., Germany) as a supplementary medication, throughout the course of the study.

Participants

The principal investigators, researchers, and clinician team members are responsible for the study design, patient screening, recruitment, conduct, and follow-up assessments during the study. All costs related to the examination, physical evaluation, stem cell administration, and medication are waived. Patient information will be protected by coding and restricting access using a computer-based system. Patients will be enrolled in the study if they meet all inclusion and exclusion criteria.

Inclusion and exclusion criteria

Patients will be enrolled in the study incompliance with the inclusion and exclusion criteria established by a screening protocol as presented below.

Inclusion criteria

- Subjects aged ≥60 and ≤85 years at the time of signing the informed consent form.
- Must show signs of frailty in addition to a concomitant condition as assessed by the investigator with a frailty score >= 3 based on the Fried Phenotype Scale.
- Showing signs of frailty based on a physician assessment in addition to a concomitant condition, as demonstrated by a score between 3 and 6 as denoted by the Canadian Study on Health Aging.
- Must provide written informed consent.

Exclusion criteria

- Mini Mental State Examination (MMSE) score less than or equal to 20.
- Active listing (or expected future listing) for transplantation of any organ.

- Clinically important abnormal laboratory values, including but not limited to the following: hemoglobin < 8 g/dl, white blood cell count < 3000/mm3, platelets < 80,000/mm3, alkaline phosphatase > 3 times the upper limit of the normal range, total bilirubin > 1.5 mg/dl.
- Serious comorbid illness that, in the opinion of the investigator, may compromise the safety or compliance of the patient or preclude successful completion of the study. This illness including but are not limited to the following: HIV, advanced liver or renal failure, class II/III/IV congestive heart failure, myocardial infarction, unstable angina, cardiac revascularization within the last six months, severe obstructive ventilator defect, COPD with GOLD D, ischemic stroke with NIHSS < 5, or type II diabetes with HbA1C >8.5%.

- Any other condition that, in the opinion of the investigator, may compromise the safety or compliance of the patient or preclude successful completion of the study.

- Be an organ transplant recipient.
- Have a clinical history of malignancy in the last five years 5 years (i.e., patients with prior malignancy must be disease-free for 5 years) with the exception of curatively treated basal cell carcinoma, squamous cell carcinoma, melanoma in situ or cervical carcinoma if recurrence occurs.
- Have a non-pulmonary condition that limits lifespan to < 1 year.

Recruitment

The patients can only enroll in this trial after completing the prescreening process, consultation resolution, and signing the informed consent form.

The patients diagnosed with frailty condition will be approached and asked to participated in the study at either Dong Da General Hospital (Hanoi, Vietnam) or Vinmec International Hospital Times City (Hanoi, Vietnam). If the patients are interested in this research, we will asked them to send the prescreen results to the administration office. A multidisciptinary consulation will be held to evaluate the prescreening results from participants to confirm the frailty condition that meet the general diagnostic criteria of frailty, including inclusion and exclusion criteria. The consulation boards will then give the final decision if more than 80% of experts agree on the prescreening results. The clinical team will set an appointment to discuss and explain in detail with the potential candidates about the clinical trial process, including advantages and disadvantages of stem cell treatments, the potential Aes

and SAEs, and sign the written informed consent form prior to assigning patients to either stem cell administration or control groups (Supplementary Figure 1).
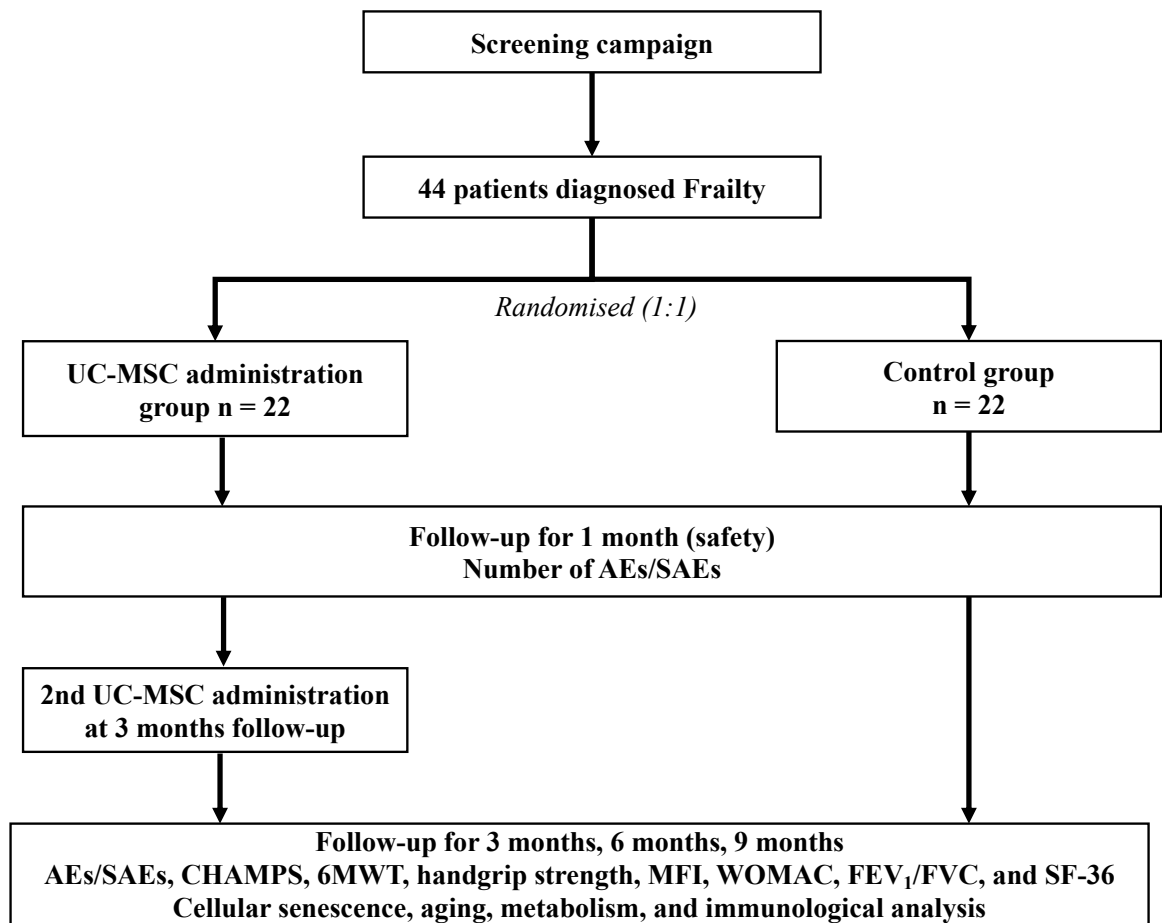
Supplementary Figure 1: Schematic of the proposed study. Elderly people will be participated in the study by enrolling in the screening campaign at Vinmec International Hospital (Times City, Hanoi, Vietnam) and National Geriatrics Hospital (Dong Da, Hanoi, Vietnam). 44 patients diagnosed with Frailty using Fried definition will be selected to the study once they meet all inclusion and exclusion criteria. All patients will be randomized (1:1) into either the UC-MSC administration group (n=22) or the control group (n=22). Patients from both groups will receive a standard medication treatment according to the Vietnamese Ministry of Health guidelines.

Intervention

A validated UC-MSC line was selected from the Vinmec Tissue Bank and cultured under xeno-free, serum-free and antibiotic-free conditions as previously described (47). To prepare UC-MSCs for therapy, aliquots of Passage 3 (P3) UC-MSCs will be thawed, cultured to P5 to obtain approximately 500 million cells and dispensed to 10 million UC-MSCs/ml/vial for cryopreservation. Upon request from the clinical team, aliquots of P5 UC-MSCs will be thawed in a temperature-controlled water bath or incubator on the infusion day. The UC-MSCs will be

washed and suspended in Ringer's Lactate solution. The thawed UC-MSC products will be accessed according to the following quality control criteria: cell viability, MSC markers, karyotype, microorganism and fungal infection, endotoxin, and mycoplasma prior to infusion into the patient at the transplantation ward (Supplementary Table 1). All patients in the UC-MSC group will receive two doses of 1.5 million cells/kg patient body weight via the intravenous (IV) route at a 3-month interval.

Supplementary Table 1: *Release criteria of cultured UC-MSC product*

Criteria	Method	Accepted criteria
Cell viability	Staining with Trypan Blue	$\geq 70\%$
Positive markers: CD 73, CD 90, CD 105	Flow cytometry	$\geq 95\%$
Negative marker: CD 45, CD 34, CD 11b, CD19, HLA-DR		$\leq 2\%$
Bacteria, fungi	Automatic culture and identification	Not detected
Mycoplasma	Bioluminescence measurement using MycoAlert® Mycoplasma kit	Not detected
Endotoxin	Chromogenic LAL Assay	≤ 0.2 EU/ kg weight for intrathecal transfusion ≤ 5 EU/ kg weight for non-intrathecal transfusion

Mode of cell adminsitration (UC-MSC group)

Patient assigned to UC-MSC administration groups will receive two administration at a dose of 1.5 million cells/kg patient body weight via the IV route with a 3-month intervening interval. On the day of administration, thawed UC-MSCs at P5 will be prepared to meet the target administration dosed based on the number of viable cells in 50 mL of Ringer Lacate (Braun, USA) as described above and delivered to the administration ward for infusion at a rate of 100 mL/hour.

Withdrawal

The withdrawal process is described previously (48). Briefly, participant discontinuation may occur upon participant death, severe adverse events (SEAs), other serious disease-limiting involvement, or a direct request from participant to withdraw from the study. Once the

participant withdraws from the study, the reasons for the withdrawal and all recorded results will be documented in detail. New participants will not be recruited to replace withdrawn participants.

Primary outcome (safety)

To evaluate the safety of the allogeneic administration of UC-MSCs for frailty, the number of adverse events (AEs) and serious adverse events (SAEs) during stem cell administration (72 h) and after 1 month, 3 months, 6 months, and 9 months will be recorded and analyzed. The AEs and SEAs of the administration of MSCs were consistent with those previously described and included death, thromboembolic event, stroke, cardiovascular abnormality, clinically significant laboratory test abnormalities, and thrombotic consequences.

Secondary outcome (efficacy)

The secondary outcomes will be evaluated to assess the effectiveness of the treatment by calculating the absolute reduction in the frailty condition. The proposed examinations were previously described and included reduced activities (using the CHAMPS questionnaire), slowing of mobility (6-min walk distance test – 6MWT), reduction of handgrip strength (dynamometer measurement), exhaustion (multidimensional fatigue inventory questionnaire – MFI), level of pain in the knee (WOMAC), respiratory function (FEV_1/FVC), and quality of life (SF-36).

To evaluate the interrelationship between the efficacy of the treatment and the UC-MSC nature and function, several molecular experiments will be planned and performed at baseline and 1 month, 3 months, 6 months and 9 months postadministration. (1) Analyses of cytokines, chemokines and growth factors in the patient plasma using cytokine/chemokine/growth factor 45-plex human ProcartaPlex panel-1 will provide information on the inflammation status and immune response of the patient to UC-MSC administration. Additionally, the secretion profiles of UC-MSCs after thawing will also be assessed using a custom-made ProcartaPlex panel that focuses on specific cytokines, such as IL-2, IL-6, IL-8, IL-10, TNF-α, VEGF, and HGF. (2) The immunoregulatory properties of UC-MSCs on the CD3+ T lymphocytes of patients will be evaluated. (3) Measurements of cellular senescence by qPCR will be conducted with the CD3+ cell population to assess the expression of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene, a specific biomarker of cellular senescence. (4) The metabolic profiles of CD3+ cells will be evaluated using the Seahorse XF Cell Mito Stress Test Kit and Seahorse XF Cell Glycolysis Stress Test Kit (Agilent Technologies).

Data collection and management

All data obtained during the study will be recorded in the patients' medical reports and the CRF, which will be checked frequently by a quality control officer in the Vinmec Times City International Hospital and Vinmec Scientific Research board for accuracy and consistency. The data in the CRF will be transferred to REDCap software within 7 days and crosschecked by the research team. The data from each patient will be collected at six time points during the course of the study, including during the screening period, at baseline and at 1 month, 3 months, 6 months, and 9 months after administration of the treatment. The data obtained during this clinical trial will be disseminated with permission from the funding body and principal investigator through national and international conferences, peer-reviewed publications, and scientific reports.

Statistical analysis strategy

Descriptive statistics will be used to illustrate the demographics of frailty in older individuals. Categorical variables will be expressed as proportions, whereas quantitative variables will be described as mean values and their standard deviations or as medians and their interquartile ranges. A t-test or Wilcoxon rank-sum test will be used to assess the relationship between the outcomes after the two infusions, whereas one-way ANOVA or the Kruskal-Wallis test will be used to assess the changes in the 6MWT, SPPB, CHAMPS, and FEV1 over time. P-values < 0.05 will be considered to indicate statistical significance. The analyses will be performed using Stata version 14 (StataCorp, College Station, TX, USA).

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Improvements and limitations

This proposed protocol will not be used in the first clinical trial using MSCs for the treatment of frailty, although it will be utilized in the first trial using UC-MSCs for the same purpose. In our study, we will manufacture UC-MSCs as an "off-the-shelf" product for the treatment of frailty. The selected source of MSCs will provide us with several advantages that overcome the challenges of several clinical trials using BM-MSCs: (1) UC-MSCs derived from perinatal sources allow their easy *ex vivo* propagation and the generation of sufficient cells for administration, (2) the active screening process of UC donors will allow the establishment of

well-characterized UC-MSC lines that meet all ISCT criteria, (3) the protocol will eliminate the threat of age- and disease-related impacts of MSC biology and function, and (4) the administration of standardized cells to all patients with frailty will allow us to measure the safety and efficacy of the treatment more precisely and reproducibly. Additionally, the UC-MSCs used in this project will be isolated and cultured in a standardized platform under xeno-free, serum-free and antibiotic-free culture conditions as previously described, will eradicate the risk of using FBS with animal-derived and unknown component material and thus, in turn, enhance the safety of cell therapy (49).

Based on the CRATUS study, we have designed a similar approach in terms of assessment of the treatment efficacy in individuals with frailty through an additional thrombotic analysis using D-dimer and CRP as two main indicators before and after UC-MSC administration, particularly at 2, 24, and 48 h postadministration. Additionally, this clinical trial is also designed such that the clinical results could be linked to the biological features of the administered product, UC-MSCs. As the mechanism of MSC actions, UC-MSCs, to be more specific, are generally related to (1) their immunoregulatory functions and (2) their secretion of cytokines/chemokines/growth factors in response to the surrounding environment (i.e., frailty condition). To evaluate the immunoregulatory functions of UC-MSCs, an immunosuppression analysis will be performed using peripheral mononuclear cells from the patients to assess the inhibitory effects of UC-MSCs on the activities of immune cells. Furthermore, the communication between the two cell types will also be captured by cytokine, chemokine and growth factor analyses of the culture medium. The alterations in the patients' immune response before and after UC-MSC administration will also be measured through the detection of proinflammatory and anti-inflammatory signals from patient plasma collected at different time points throughout the course of the study. Measuring the improvements in the patients' cellular aging process is difficult due to the complexity of the disease condition. In this trial, we attempt to indirectly assess the reduction in the aging process induced by UC-MSC administration by measuring (1) the immunological composition, (2) cellular senescence of CD3+ cells from peripheral blood, and (3) metabolic profiles of CD3+ cells via a Seahorse XF Cell Mito Stress Test Kit and Seahorse XF Cell Glycolysis Stress Test Kit (Agilent Technologies).

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	9-10
Participants	4a	Eligibility criteria for participants	9-10
	4b	Settings and locations where the data were collected	8, 10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	10 – 11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	11-12
	6b	Any changes to trial outcomes after the trial commenced, with reasons	8
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	11, 12
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Not applicable

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Not applicable
	11b	If relevant, description of the similarity of interventions	Not applicable
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	13

Results

Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10,11
	13b	For each group, losses and exclusions after randomisation, together with reasons	10,11
Recruitment	14a	Dates defining the periods of recruitment and follow-up	10,11
	14b	Why the trial ended or was stopped	Not applicable
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Not applicable
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8,9 Figure 2 (main paper)
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11, 12, 13
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	11, 12, 13
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	11, 12, 13
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11, 12, 13

Discussion

Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13, 14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14

Other information

Registration	23	Registration number and name of trial registry	8
Protocol	24	Where the full trial protocol can be accessed, if available	Not applicable
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.